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AUTHORITY

BDRL ltr, 13 Sep 1971

(1) Cy

TRANSLATION NO. 25

DATE: July 1968

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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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ELECTRON MICROSCOPE by. G. Kellenberger.

Preparation of the Biological Object Through "Filtration of Agar"

Principle:

Suspension

5 drops are spread with a flat glass plate

Petri dish

Collodian membrane

With 15% difac-bacto agar, solidified solution suspension

After filtration-fixing in formalin vapor

An agar block is pitted and the colloidian membrane washed off with a solution of 2% Lanthan-Nitrate.

The introduction of water on the surface is prevented by the combination Formalin - $LaNO_3$.

The floating membrane is picked up on the carrying screen from underneath.

By this method of preparation we will avoid:

a. Drying (high salt concentration) by immediate sealing of the petri dish; with delicate objects the expansion is undertaken in a humid air chamber.

b. Changing of the suspension media by assimilation of the agar-base and the solution which is present.

c. Re-moistening of the object which is floating by addition of Lanthan-Nitrate after formalin fixation; which combination prevents the introduction of water to the colloidian surface.

d. Selection: all undialysable material are found in the colloidian membrane.

Executive and practical limits

a. Producing the agar plates: To a suspension solution of about 30% distilled water is added and to this diluted solution 15 gr. of Bacto-agar per liter is added. It is sterilized in an autoclave 20-30 cc poured into each petri dish. After solidification the plate is dried until the previously added distilled water is lost (30%). (After the initial 30-60 minutes the controls are kept constant by considerations, conditions, and agar quantities.) Thus the surface of the agar becomes strongly absorbent without changing the salt concentration in relation to the given suspension media.

b. Producing the colloidian membrane: The agar plates are purified of all traces of fat before or still better after drying by pouring over distilled petroleum ether and are so arranged that the place of contact of the finger on the outer rim of the plates in the course of manipulation remains marked and in the process of pouring the colloidian solution from plate to plate does not come into contact with the fluid. The collodian solution (0.2% in distilled amylacetate) is now poured from plate to plate, which are being turned over the agar. The petri dishes are then reversed (open) for 6 hours and dried on a fat free substrate.

Minimal traces of fat make filtration impossible!

c. Filtration: 5 drops of the suspension are carefully spread with a glass rod (it is best for one to hold the glass rod in the drops and draw out the dish). In 10-20 minutes filter the thin fluid like section through the collodian. When this does not occur the membrane is stopped up by traces of fat and is unusable for filtration: the suspension dessicates. Bacterial suspensions filter rapidly very well; bacterial lysates filter more slowly.

Bacteriophages are purified in part by centrifuging and redissolving in a fresh medium. For synthetic nutrient solutions one must add a wetting agent (for example pepton) to the spread. The best control whether a filtration occurs or whether the solution only dessicates is shown by the results and will be discussed there.

d. Fixation: immediately after the end of filtration (eventually places which are still damp can be noted on the opening of the dish and separated) the plates are fixed in formalin vapor by laying the reversed plates on a vessel which contains 40% formalin for 7 minutes. The formalin fixation is indispensable in all cases where one must prevent the entrance of water into the floating substance - it appears that in this connection only the combination formalin fixing - lantannitrate is reliable. Fixing in OsO_4 vapor is employed for the demonstration of nucleodes in bacteria with subsequent floatation by distilled water (see under results, Section e).

e. Floatation: a small block is "injected" with a spatula of agar, dipped obliquely into a 2% solution of La-Nitrate (Merck) and the easily perceptible floating membrane is rapidly hooked from underneath with a carrying rack and dried well between two blotting papers under heave pressure. The preparation could be further dried under a vacuum. In the manner innumerable preparations could be made per plate; one prepares with profit at least two different areas of the petri dish.

Results and discussion - Part d only

d. Other biological objects, bacteriophages: It was not said before that all suspensions are suited for filtration from agar and this must be studied for each individual case. We have established that the method is excellently suited for clean phage preparations and the latter method reproduces just as well as for example the congealed-drying method (Williams). However as soon as the solution dries instead of filtering it, rupture the phages in the film. We have also employed the method with success for preparations of muscle fibers from a dilacerat.

In conclusion we should repeat that filtration from agar is a relatively simple and rapid method of preparation to run; which works with great accuracy with the objects until the moment of fixing with the condition, however that one prepares a collodian which really filters. The foregoing will be fulfilled with greatest care relative to fat traces in the collodian solution on the plate. (Be careful of drops on the flash, finger prints, pre-washing of the paradian in the solid state, etc.)

April 1954

G. Kellenberger

Institute of Physics
Dept. of Biophysics
Univ. of Geneva

Translated from German by

C. Robins